ORIGINAL ARTICLE

Detection of voluntary dehydration in paediatric populations using non-invasive point-of-care saliva and urine testing

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Aim: Voluntary dehydration, or lack of fluid intake despite water availability, is common in otherwise healthy children, and can lead to adverse effects. Most dehydration biomarkers are impractical for routine assessment in paediatric populations. This study aimed to assess two non-invasive hydration assessment tools, urine specific gravity (U_{SG}) and a novel point-of-care (POC) salivary osmolarity (SOSM) sensor, in healthy children.
Methods: Volunteers were tested by colorimetric U_{SG} and a handheld SOSM system. Observed values were compared against previous studies to determine hydration status, as was the concordance between parameters.
Results: At the common U_{SG} threshold of 1.020, 42.4% of the 139 healthy children were dehydrated. The same prevalence was found using the 70-mOSM cut-off value. Comparative analysis of SOSM at varying U_{SG} thresholds demonstrated significantly higher SOSM in dehydrated children with a $U_{SG} \geq 1.030$ ($P = 0.002$).
Conclusion: At the U_{SG} threshold of 1.020 and SOSM threshold of 70 mOSM, 42.4% of healthy children were found to be voluntarily dehydrated. Significantly higher SOSM was observed in dehydrated children ($U_{SG} \geq 1.030$). As the first study on the utility of POC SOSM measurements for detecting dehydration, these results provide a foundation for future POC characterisation of SOSM in other populations and clinical contexts.

Key words: point-of-care; salivary osmolarity; urine specific gravity; voluntary dehydration.

What is already known on this topic:

1 Voluntary dehydration is common in otherwise healthy children.
2 Dehydration in children can impact both physiological and cognitive processes.
3 Due to the subtlety of physical dehydration signs, detection often still rely on urinary and plasma parameters, which may be impractical for everyday use.

What this paper adds:

1 This is the first study to report the utilisation of a novel method of detecting dehydration in children, using a point-of-care saliva osmolarity (SOSM) biosensor.
2 When benchmarked against a commonly-used Urine Specific Gravity (U_{SG}) threshold of 1.020, and SOSM threshold of 70 mOSM, 42.4% of healthy children were found to be voluntarily dehydrated. Significantly higher SOSM was observed in dehydrated children ($U_{SG} \geq 1.030$).
3 These results provide a foundation for future point-of-care characterisation of SOSM in other populations and clinical contexts.

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Water is the major constituent of the human body, and hence maintenance of fluid balance is essential for metabolic integrity and the support of physiological functions. In infants, water makes up 75% of the total body, with a steady reduction during childhood, reaching the adult body water composition of 60%. As the majority of fluid intake originates from food and beverages, young children often require parental or guardian encouragement to ensure adequate fluid intake. The higher water composition in children, together with the tendency to depended on adult encouragement, places the paediatric population at higher risk of suffering from dehydration or excessive water loss.[1]

Voluntary dehydration, or lack of fluid intake despite the presence of adequate liquid sources, has been shown to be more prevalent in various temperate regions.[2] Although not conclusive, there is evidence to suggest that voluntary dehydration can impact physiological and cognitive processes in children. In infants, voluntary dehydration has been associated with confusion, irritability and lethargy, whereas in adolescents the condition has been shown to cause a decrease in cognitive performance.[3] Dehydration in otherwise healthy children has also been shown to affect visual ability and memory.[4]

While there is no gold standard for routine assessment of dehydration, several parameters have been proposed. These include clinical assessment, plasma osmolarity, plasma electrolyte concentration, urine osmolarity, urine colour, urine specific gravity (U_{SG}) and salivary osmolarity (SOSM).[5] Physical characteristics of voluntary dehydration can be subtle and often go undetected, and so while physical diagnosis is routinely applied for pathological dehydration, such as in diarrhoea, it lacks sensitivity and specificity for cases of voluntary dehydration and/or the objectivity for routine use by general health-care workers, parents and teachers who may wish to assess the hydration status of a child. While plasma biomarkers are considered highly accurate, they are both invasive and impractical for routine application in otherwise healthy children.

Urinary measures are commonly used for assessing hydration status in research, clinical, industrial and athletic contexts due to the less invasive nature of collection. One of the most commonly used urinary parameters for determining hydration status is U_{SG}, although there is a lack of consensus of how dehydration should be defined using U_{SG}. The simplest tool for the assessment of U_{SG} is a colorimetric test strip, whereby a strip is briefly submerged in a urine sample and a colour change is observed. Commonly used U_{SG} cut-off values for dehydration range between 1.020[6–9] until 1.025[10,11] and 1.030[12,13] with higher cut off resulting in higher specificity.[14]

A recently proposed alternative method of hydration assessment is measurement of SOSM, defined as the concentration of solute particles, predominantly electrolytes, per litre of saliva. Previous studies have characterised the use of SOSM in both adults and children, using a laboratory-based freezing point osmometer.[15,16] Multiple studies have shown the accuracy of spot-check SOSM for determination of hydration status both in sport as well as clinical applications.[15,17–20] However, despite the correlation between SOSM and other laboratory-based dehydration parameters, real-world routine application has been hindered by the logistical boundaries associated with use of benchtop osmometers.

An ideal assessment technology for dehydration should be accurate, easy to use, rapid, non-invasive and pleasant for both user and operator.[21,22] Recently, a novel point-of-care (POC) salivary osmometer has been developed for use in athletic and health and safety contexts. The platform is rapid, non-invasive, does not require laboratory equipment or qualified experts to perform the measurements and has been shown to report values highly correlated with a benchtop osmometer ($r = 0.96$).[23] In this study, we describe the use of a U_{SG} test strip and a novel, saliva-based, POC osmometer for the detection of hydration status in a paediatric population. We compare the observed values against previously described reference ranges for U_{SG} and SOSM to assess voluntary dehydration and the concordance between hydration assessments with these technologies. This study seeks to establish reference values of paediatric hydration status using SOSM, as a basis for future studies on the level of dehydration in disease states.

Methods

Research participants

The study was conducted in the city of Makassar during the dry season in February–June 2019. This study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine, Hasanuddin University (Ethics number: 150/UN4.6.4.5.31/PP36/2019). Ethical clearance was obtained from the Medical Research Ethics Committee prior to commencement of the study. The age criteria for inclusion were based on the World Health Organization definition for children as individuals aged between 0 and 18 years old. The sample population consisted of healthy child volunteers from several housing areas in the city of Makassar. The participants were sampled while doing their usual activities and were only instructed not to eat or drink 30 min before the examination. All sampling was performed between 16.00 and 18.00 pm. Before participating in the study, written consent was obtained from parents or guardians of the children following a thorough explanation of the study protocol. Participants were excluded in the presence of factors and conditions that may affect hydration status such as gastrointestinal symptoms (diarrhoea, nausea and vomiting), fever and pharmacological intervention with diuretics or pre-existing disease (e.g. renal impairment).

Hydration measurements

U_{SG} testing

The test was conducted as a spot-check performed during the late afternoon. Each child was issued with a urine sample container labelled with a unique identification code. Subjects were asked to provide a mid-stream urine sample, independently or with parental/guardian assistance. Upon sample return, U_{SG} was measured using the Dirui H10 urinalysis strip (Dirui, Istanbul, Turkey).

POC SOSM testing

Saliva samples were obtained at the same time as U_{SG} measurements using a novel and portable POC saliva osmometer (MX3 Diagnostics Inc., Melbourne, Australia; Fig. 1). The osmometer utilises disposable pre-calibrated biosensors to determine SOSM based on electrochemical impedance spectroscopy, with quantitative digital output of SOSM values. Saliva sampling was performed with the prerequisite of no eating or drinking 30 min prior to the measurement. Sampling was performed in triplicates for all subjects, by tapping the pre-calibrated sensor strips directly on the child's tongue for several seconds until the osmometer registered that a sample had been collected. The average value of the triplicates was used for analysis across all subjects.
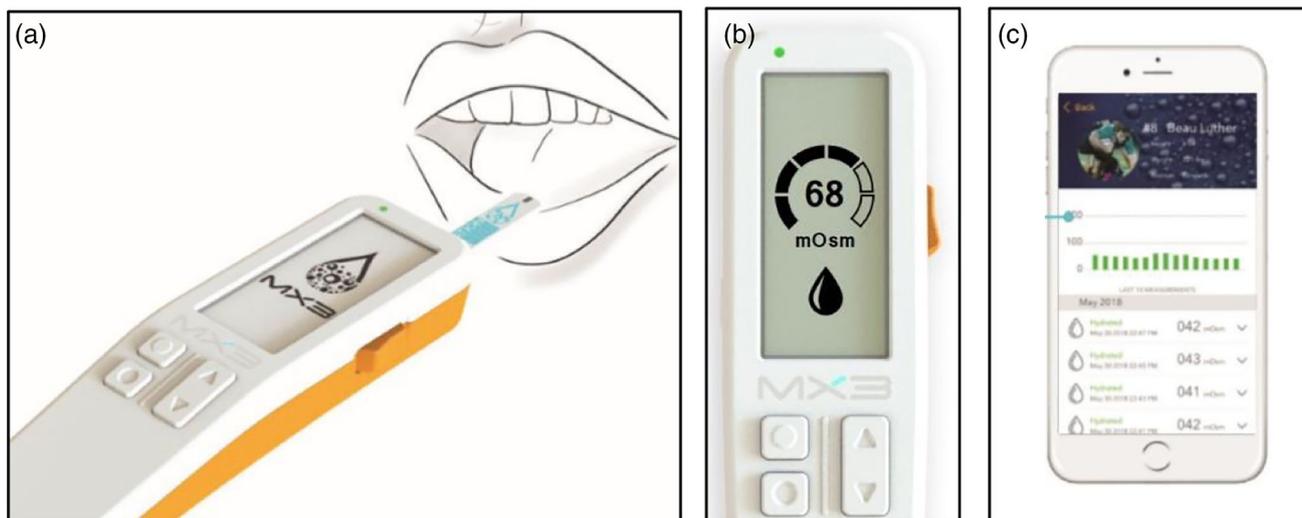
Fig 1 Portable point-of-care saliva osmometer. (a) Saliva was sampled using pre-calibrated, disposable sensor strips. (b) Quantitative output of salivary osmolarity was displayed on the reader interface. (c) Real-time and systematic data collection was achieved through an integrated App supported on an iOS operating system.

Table 1 Sample characteristics based on gender

Sex	Number of samples (*n*)	U_{SG}	SOSM
Female	80	1.016 ± 0.007	67.21 ± 14.06
Male	59	1.017 ± 0.008	69.36 ± 16.81
Total	139	NA	NA
P value	NA	0.1535	0.4784

Results are shown as mean ± standard deviation. NA, not applicable; SOSM, salivary osmolarity; U_{SG}, urine specific gravity.

Table 2 Sample characteristics based on age group

Age group	Number of samples (*n*)	U_{SG}	SOSM
≤5 years	30	1.016 ± 0.008	66.90 ± 14.56
6–10 years	90	1.017 ± 0.007	69.14 ± 16.08
>10 years	19	1.017 ± 0.009	65.21 ± 12.24
Total	139	NA	NA
P value	NA	0.9257	0.5289

Results are shown as mean ± standard deviation. NA, not applicable; SOSM, salivary osmolarity; U_{SG}, urine specific gravity.

Table 3 Prevalence of voluntary dehydration in the healthy child population based on various salivary osmolarity (SOSM) and urine specific gravity (U_{SG}) thresholds

Prevalence of dehydration in healthy children based on common U_{SG} cut off values	
U_{SG} threshold	Dehydration prevalence
≥1.020	59 children (42.4%)
≥1.025	36 children (25%)
≥1.030	11 children (7.9%)

Prevalence of dehydration in healthy children based on several SOSM cut-off values	
SOSM threshold	Dehydration prevalence
≥70 mOSM	59 children (42.4%)
≥80 mOSM	31 children (22.3%)
≥90 mOSM	7 children (5%)

Results are shown as mean ± standard deviation.

Statistics

Microsoft Excel (Microsoft Corporation, Redmond, Washington, US) and GraphPad Prism (GraphPad, San Diego, California, US) Version 8.1.2 were used to generate tables, graphs and perform statistical analysis, with the significance threshold set at a *P* value of 0.05. Descriptive statistics were used to show sample characteristics. The Mann–Whitney *U* test was used to assess the differences between two groups. Differences in U_{SG} and SOSM between age groups were tested with Kruskal–Wallis analysis of variance. Correlation between U_{SG} and SOSM was assessed with Spearman's rank correlation test. A receiver-operating characteristic curve was generated for each of the three cut-off values to determine the largest area under the curve (AUC) for SOSM, and to calculate the sensitivity and specificity of an optimal SOSM cut-off value. Cohen's kappa coefficient was calculated using GraphPad QuickCalcs (GraphPad, San Diego, California, US) to
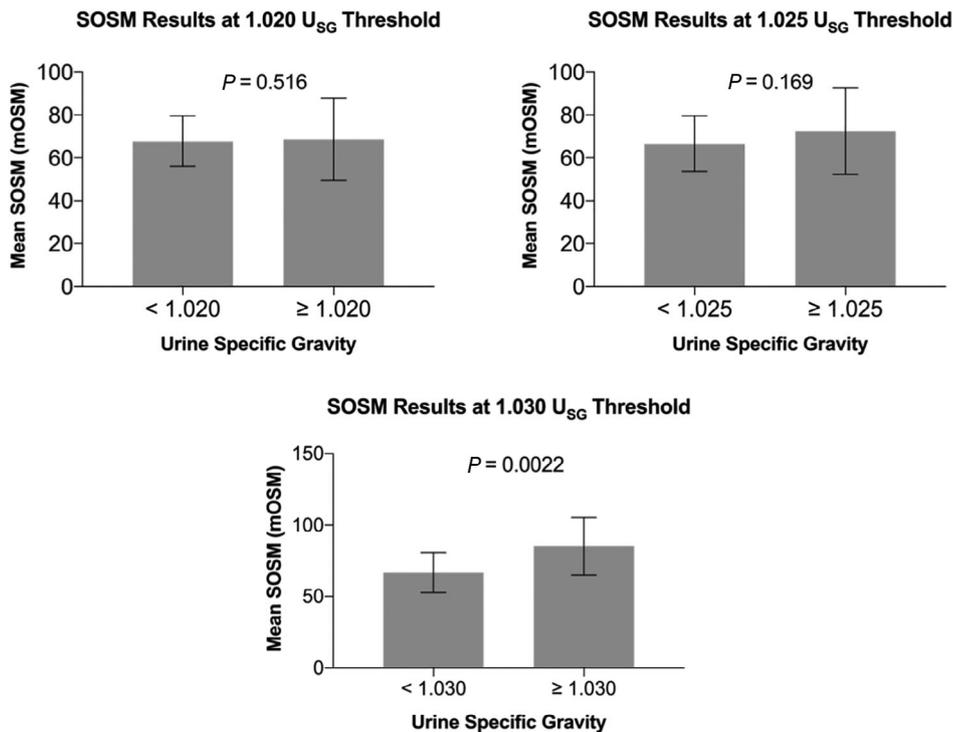
SOSM Results at 1.020 U$_{SG}$ Threshold

SOSM Results at 1.025 U$_{SG}$ Threshold

SOSM Results at 1.030 U$_{SG}$ Threshold

Fig 2 Comparison between the salivary osmolarity results of the healthy child population at urine specific gravity cut-off value of 1.030. Mean ± standard deviation values are shown, with *P* value obtained through the Mann–Whitney test.

quantify agreement between three U$_{SG}$ thresholds with arbitrary SOSM thresholds.

Results

Sample characteristics

In total, samples from 139 healthy child volunteers were obtained from various districts in the city of Makassar. The sample population consisted of 59 males and 80 females, with ages ranging from 1 to –14 years (median age = 8 years). The average SOSM of this paediatric population was 68.12 ± 15.27, with no significant difference between male and female children (67.21 ± 14.06 and 69.36 ± 16.81, respectively; Table 1). No significant difference in SOSM was also observed across the age groups (*P* = 0.5289) (Table 2). Meanwhile, the average U$_{SG}$ of this paediatric population was 1.016 ± 7.8, with no significant difference between the male and female children (average U$_{SG}$ = 1.017 ± 0.008 and 1.016 ± 0.007, respectively; Table 1). Similarly, no age-group based difference was observed in the U$_{SG}$ measurement (*P* = 0.9527) (Table 2).

Dehydration based on several SOSM and U$_{SG}$ cut-off values

We then determined the prevalence of dehydration in this sample population based on several commonly used U$_{SG}$ thresholds of 1.020, 1.025 and 1.030 (Table 3), yielding a dehydration prevalence of 42.4, 25 and 7.9% of children, respectively. As no consensus is currently available regarding the dehydration threshold of SOSM in a paediatric population, we determined the prevalence of dehydration based on several SOSM thresholds (Table 3)

of 70, 80 and 90 mOSM. These cut-off values yielded a dehydration prevalence of 42.4, 22.3 and 5%, respectively.

As shown in Table 3, both methods revealed relatively similar numbers of voluntarily dehydrated children in this healthy paediatric population. And although the correlation between SOSM and U$_{SG}$ as a whole (in both dehydrated and hydrated children) was poor (*r* = 0.02 and *P* = 0.73; Fig. S1, Supporting Information), a comparative analysis of SOSM at varying U$_{SG}$ thresholds demonstrated that a significantly higher SOSM was observed in dehydrated children with a U$_{SG}$ ≥ 1.030 (85.27 ± 20.16 vs. 66.65 ± 13.91 mOSM, *P* = 0.0022 Mann–Whitney *U* test; Fig. 2). We also confirmed the higher agreement between the SOSM platform in highly dehydrated individuals (U$_{SG}$ ≥ 1.030) using receiver-operating characteristic analysis (Fig. S2, Supporting Information), showing a higher AUC value of SOSM testing (AUC = 0.77) when benchmarked against the highest U$_{SG}$ cut-off value relative to the two lower U$_{SG}$ cut-off values. At the cut-off value of 70.5 mOSM, the POC saliva test yielded a sensitivity and specificity of 81.82 and 64.06%, respectively. In addition, the threshold of U$_{SG}$ ≥ 1.030 also resulted in the most optimal Kappa coefficient (*k* = 0.4; Table S1, Supporting Information). This indicates that although the two methods lack agreement in hydrated individuals, there is strong concordance and discrimination capacity for the identification of highly dehydrated individuals.

Discussion

The challenge of determining subclinical hydration status in otherwise healthy children is the lack of clear physical signs, and the impracticality of invasive gold standard measures for routine assessments. In this study, we focused on assessing the presence of voluntary dehydration among a population of otherwise healthy

children using two different types of non-invasive methods, SOSM and colorimetric U_{SG} testing. U_{SG} has been used routinely for assessment of dehydration, although no clear consensus exists regarding an effective cut-off value for dehydration. The relatively new SOSM approach may be more favourable due to the less invasive nature of sampling in comparison to urinary tests, although previous characterizations have been performed using laboratory-based benchtop osmometers,[15,16] which are not readily available in most laboratories in Indonesia. To our knowledge, this is the first study to demonstrate the potential of a POC osmometer for routine hydration assessment in a paediatric population.

Due to the lack of clear consensus for hydration assessment with U_{SG}, we determined the prevalence of dehydration using three common cut-off levels. At the lowest common cut-off value of 1.020, it was found that 59 (42.4%) out of the 139 healthy children were dehydrated. The same prevalence was found when we used a cut-off value of 70 mOSM with the SOSM measurement. This is in line with the study conducted by Bonnet *et al.*, which suggested that two-third of healthy school-aged children in France were dehydrated despite regular food and beverage intake.[24] Other studies have shown that this type of voluntary dehydration is more likely to be moderate to severe in children living in hot and dry environments.[2] This becomes important to note because voluntarily dehydrated children often do not have any complaints so that parents and guardians do not realise that their children are dehydrated.

Several factors may have contributed to the poor correlation between U_{SG} and SOSM seen in our study in circumstances other than overt dehydration. First, urine spot-checks can be unreliable, being affected by multiple factors including diet, recent fluid intake and physical activity.[6,11,25–28] In addition, the interpretation of colorimetric test strips can be subjective,[29] although this may be addressed by using an automated reading of the strips.[30] In future studies, this correlation may be improved through assessment of U_{SG} in first-morning urine to minimise interfering factors when trying to establish the concordance between the two methods. In addition, assessment of U_{SG} using refractometers may improve the precision and accuracy of U_{SG} measurements,[25] although the impracticality of this equipment may preclude routine use by parents and caregivers.

There were also several limitations associated with the POC SOSM method used in this study. First, we limited fluid and food intake prior to measurement, which would be impractical in real-world contexts.[10] However, while we opted for waiting at least 30 min after any food or drink were consumed to conduct measures, others have reported that the effect of oral intake is not seen after 15 min from an oral rinse and hence there is potential to reduce the duration of this embargo in future studies.[31] Another limitation is the presence of normal inter-individual variability of the SOSM measurement in users not accustomed to producing saliva for sampling, which was accommodated here by measurement of triplicates. Moreover, there remains a possibility that impedance measurement may also be affected by several irrelevant matrices within the saliva, which cannot be removed by restraining consumption of food and drinks 30 min prior to sampling. Lastly, while one recent study found SOSM measurements to be the most effective ancillary tool for assessing hydration status of elderly patients when benchmarked against plasma osmolarity, there are limited data assessing the utility and interpretability of spot-check measurements in other clinical populations.[19]

Although SOSM testing was generally more favourably received than urine sample collection by children, parents and guardians, in several children sampling proved to be challenging when the child was uncooperative or unable to provide enough saliva in order to obtain a complete reading. Moreover, during sample collection, we were frequently unable to obtain samples from passive drooling, instead utilised directly from tongue sampling which was easier for the young children. This may reduce the performance of the POC SOSM test, based on an independent validation study that showed a stronger correlation ($r = 0.96$) between the POC SOSM with benchtop osmometers when samples were obtained through passive drooling, relative to a moderate correlation ($r = 0.77$) when samples were obtained directly from the tongue.[23]

With both methods utilised in this study, lower threshold values suggest that a large portion of children are dehydrated, at a number (~40%) that is in line with previous studies that utilised urine-based hydration measurement parameters, which show a 37%[2] and a 54.5%[32] prevalence of dehydration. However, despite the said concordance, it is also worth noting that recent studies have shown that spot-checks at lower U_{SG} thresholds (≥1.020) can greatly overestimate the number of dehydrated subjects.[6]

The population of interest itself may be a crucial variable that needs to be considered when determining dehydration thresholds using SOSM. When benchmarked against plasma values, previous studies have reported an average SOSM of approximately 65 in euhydrated young adults, increasing to approximately 90 at 1% body mass loss (BML) and approximately 130 at 2% BML induced by exercise,[18] This differs from the findings in elderly patients which show an average SOSM of 92 mOSM among euhydrated patients and approximately 140 among dehydrated patients.[19] This suggests that threshold value may be dependent on the population of interest as well and that lower thresholds of <90 may be indicative of a mild dehydration (1–2% BML) – which, while not compromising physical health, is associated with increased fatigue and impaired memory and mood state.[33]

Despite the current limitations, this study has provided the preliminary data from a healthy child population to establish reference values of paediatric hydration status based on SOSM. This can be used as a basis for future studies on the level of dehydration in both physiological or disease states of both children and adults, such as paediatric diarrheal diseases, hydration monitoring during use of diuretic medications or monitoring of hydration in pregnancy. We predict that the ease of operation, high speed and accuracy of this testing platform will allow simpler means for long-term and continuous monitoring of hydration status.

Conclusion

The number of healthy children found to be voluntarily dehydrated was 42.4% when using the common colorimetric U_{SG} dehydration threshold of 1.020. The same prevalence was found at an SOSM threshold of 70 mOSM. Significantly higher SOSM was observed in dehydrated children with a $U_{SG} \geq 1.030$. As the first study on assessing POC SOSM measurements for detecting dehydration in a paediatric population, these results provide preliminary evidence for the utility of SOSM as a separate and complimentary metric and lay a foundation for future

POC characterisation of SOSM in various other populations and clinical contexts.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Overall and threshold-specific correlations between salivary osmolarity and colorimetric urine specific gravity.

Fig. S2. Receive-operating characteristics curve of salivary osmolarity measurements benchmarked against urine specific gravity dehydration cut-off values.

Table S1. Kappa statistics for the agreement between three commonly used urine specific gravity cut-off values and three arbitrary salivary osmolarity cut-off values for the classification of dehydration in the normal child.